Middle-East Journal of Scientific Research 21 (2): 358-361, 2014
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2014.21.02.21398

Effectiveness of the Multi-Substrate Testing (MST) While Identifying the Soil Pollution with Heavy Metals

Bahyt Nasyrovna Mynbayeva, Bahitkul Kenesovna Amirasheva, Imanbekova Tamara Galymzhanovna and Laura Kenesovna Amirasheva

Kazakh National Pedagogical University Named after Abai,
Dostyk Av., 13, Almaty, 050010, Kazakhstan

Abstract: Three different methods were used for studying the ecological state of the soil in Almaty city: chemical method, microbiological method and multi-substrate testing (MST). Microbiological methods can be used for assessment of the microbial community state in the soil ecosystem, but they take a lot of time and efforts and are less informative. Chemical method can define only the level of heavy metals content in the soil and suppose the suppression of pedobionts in their presence. At using the MST the main characteristic of the soils state was the rate of recycling of nutritious substrates by the soil bacteria. The analysis of the multidimensional indices of the bacterial community reaction of the city soils allowed us to consider it as biosensor. With the help of MST we got the integral reaction of the bacterial community biological activity which was reducing in the presence of heavy metals. Thus, the profile of the substrates assimilated by the soil microbial flora can serve as the indices for the state of communities and soils altogether. The used MST method measured structural and functional parameters of the ecosystem through the use of organic substrates by the communities for assessment of the soil medium quality. We can also estimate the degree of bacterial communities deterioration influenced by heavy metals.

Key words: Bacterial community · Urbanozems · Heavy metals · Chemical method · Method of multi-substrate testing

INTRODUCTION

One can estimate the soil state through the study of soil microbial communities which are used as indicators of deterioration processes of ecosystems. However these methods take a lot of efforts. The use of chemical methods allows one to state the level of soil pollution by pollutants, in particular by heavy metals (HM). However there are disadvantages of chemical methods which limit their use [1]; it is especially difficult to assess actual danger and forecast the consequences of HM influence of living organisms.

It was stated that soil pollution with HM and their compounds had negative effect of the vital activity of microorganisms and general ecological state of the soils [2, 3 etc.].

The study of microbial communities for one substrate is a less informative method for revealing functional links in the soil coen [4]. The use of the great range of organic substances and reactivity of microbial communities for them was implemented in the first decade of the XXI century on the basis of modern methods of registration and computerized processing of multi-substrate data. Later this method was called multi-substrate testing (MST) of the soil microbial communities. The basis of this method is the analysis of profile of consumption of substrates by the microbial communities. For studying the soil state of Almaty city we used the system of MST "Ekolog", proposed by the Russian scientists [5], according to the consumption rate of test pattern of organic mono-substrates (in the quantity of 47) during the incubation in the special test titer plates.

Corresponding Author: Bahyt Nasyrovna Mynbayeva, Kazakh National Pedagogical University Named after Abai, Dostyk Av., 13, Almaty, 050010, Kazakhstan.

The assessments are made on the photometric basis using the indicator of dehydrogenase activity of bromic triphenyl tetrazolium.

Work objective: use of the modern method of multi-substrate testing (MST) of the soils and comparing its effectiveness with other methods.

In 2011 we used the MST method for assessment for 5 sections of soil mantle in Almaty city with the help of the staff of the soil biology chair of the soil sciences department of Lomonosov Moscow State University [6]. In 2013 soil samples were taken on the other sections; the investigations were carried out in the biomonitoring laboratory of the Abay Institute for master program and PhD of Kazakhstan National Pedagogic University on the acquired equipment "Ekolog".

Objects and Methods of Investigation: We investigated the samples of the upper level of the soil mantle in Almaty city on the depth of 0-20 cm; the samples were taken on the following areas: T.1 - Abay avenue / Seyfullin avenue, T.2 - airport and T.3 - park belt of the Kazakhstan National University (KazNU). Consequently the first two sections of sampling were polluted by urbanozems and the park belt which is not subjected to the direct influence of man-made emission was chosen as a control sample.

The preparation of soil samples and measurement of heavy metal content (Pb, Cd, Cu, Cr and Zn) were performed after pouring of 5 ml 5M HNO_3 and 3 ml H_2O_2 to the 2 g of soil, then after selecting the programme which corresponded to the quantity of samples, the samples were subjected to mineralization. After the completion of the programme the samples were cooled and filtered. The measurements were performed on the spectrometer «Shimadzu» with electrothermal atomization AA-6650 [7].

Structure and quantity of microbiot was investigated by means of ordinary microbiological methods [8, 9].

Sample for MST were represented by the representational mixed soil sample. 100 ml of phosphate buffer (pH 6,5) are poured into the glass with soil weigh, then it is processed by ultrasound (УЗДН-1, 22 kHz, 0.04 A) during 30 seconds. The suspension (40 ml) was centrifuged with the help of MiniSpin, 2000 r/min during 2 minutes. 2 ml of bromic triphenyl tetrazolium solution are added to 20 ml of supernate.

Standard titer plates for immunological tests were used for MST. 200 mcl of a certain substrate of 47 offered ones (including sugars, alcohols, organic acids salts, aminoacids, amines, amides, nucleosides) are added to every cell, then there were added the set of mineral salts, bromic triphenyl tetrazolium and 0.2 ml of soil suspension. Then it was incubated in the temperature regulator up to 72 hours (depending on the time of appearing of visually registrable colour) at the temperature +28°C.

The recovery of non-coloured salts of bromic triphenyl tetrazolium to vinous-coloured formazan took place under the condition of biological activity of bacteria in relation to the utilization of some substrates. After the incubation the registration of MST data by the hardware-software complex "Ekolog" was performed. Optical density of cells of a plate was measured with the help of plate spectrophotometer (analyser "Uniplan") within the range of 510 millimicron, biodiversity parameters and order distribution coefficient of substrate consumption profiles were calculated automatically.

Body: Investigation Results: Maximum content of *Azotobacter* bacteria and soil yeasts was observed in reference soil samples, minimum content was observed in urbanozems of the road crossing with a significant content of HM. The number of free-living fixers was 1.8 and 1.4 times reduced (correspondingly) in urbanozems comparing to the blank sample (Table 1).

We determined the increase of cryptogamic bacteria quantity (for 32%) and Actinomycetes quantity (for 28%) in urbanozems from T.1 comparing to the reference soil (Table 1). The quantity of microscopic fungi was 2.9 times higher in soil samples from T.1 and 1.3 times higher in samples from T.2 comparing to the reference soil of T.3, which can be explained by the stability of micromycetes to the high concentration of HM in soils (Table 2).

Thus, the use of microbiological methods of soil state assessment in Almaty city allowed us to get information about microbiological consequences of soil pollution with HM: it was noted that they influence the content of soil microbiot; the quantity of micromycetes, cryptogamic bacteria and actinomycetes increased in urbanozems; the quantity of nitrogen-fixing bacteria and soil yeasts sensitive to HM reduced. However, comparing the efforts spent for microbiological experiments with the accuracy of the acquired data on quantity (calculation error is approximately 30%) it is obvious that the assessment of the actual impact of HM on phenotypic biodiversity of microbial communities and moreover on their quantity by culture techniques can be incorrect since the soil samples could contain uncultivated microbial strains or microbial strains which do not grow in the given medium.

Table 1: Quantity of some main groups of microbial flora of soil in Almaty city

Area of sampling	*Azotobacter* (%)	Yeasts (%)	Bacteria, $\times 10^6$CFU/	Actinomycetes, $\times 10^5$CFU/g	Fungi, $\times 10^4$CFU/g
T.1	54.14±0.88	52.8±0.17	2.07±0.31	8.10±1.84	2.77±0.29
T.2	71.67±1.20	58.8±0.15	1.68±0.46	5.20±1. 04	1.23±0.16
T.3	98.33±1.74	94.2±0.53	1.40±0.40	2.30±0.85	0.95±0.18

Table 2: Content of HM in the soils of Almaty city

	Concentration of HM, annual average, mg/kg				
Area of sampling	Cd	Pb	Cu	Cr	Zn
T.1	0.2±0.06	30.2±6.04	2.1±0.63	2.6±0.65	21.4±4.71
T.2	0.4±0.09	53.9±10.2	5.7±1.3	1.0±0.28	29.5±5.9
T.3	0.2±0.07	32.3±6.78	3.0±0.81	3.7±0.85	20.3±5.28

Table 3: Substrates in the test titer plate "Ekolog" utilized by the bacterial community of soil samples of Almaty city

Area of sampling	Qoantity of substances from the SES	Designation of the consumed substrates
T.1	9	L + arabinose, citrate, aminoethanole acid, proline, histidine, galactose, serine, L - arginine, fruit sugar
T.2	18	L + arabinose, L + isodulcite, lactose, D-mannitol, D+maltose, D+glucose, aspartate, citrate, succinate, maleate, aminoethanole acid, proline, ribose, alanine, asparagine, L-arginine, amylum, mellitose
T.3	30	Inose, L+arabinose, dulcite, D+sorbite, D+glucose, saccharose, xylose, aspartate, citrate, succinate, aminoethanole acid, proline, ribose, galactose, mannose, histidine, threonine, alanine, asparagine, valine, serine, lactate, L-glutamine, L-arginine, L-lysine, thymidine, amylum, fruit sugar, raffinose, glycerin

Table 4: Characteristics of sampling areas of Almaty city acquired by the MST method.

Area of sampling	Parameter d	Description of a system
T.1	0,9	Critical destabilized system
T.2	0,4	System with depleted resources or system under the reversible influence of some disturbing factor
T.3	0,05	Satisfactory redundant system with the maximum safe coefficient

Table 2 represents data on the content of HM in soil samples of T.1-3 that were acquired by the chemical (spectral) method.

It is possible that the soil samples could have other metals, but our capabilities were limited by the determination of 5 HM since chemist-analysts had only these samples of HM. The error of this analysis is approximately 20-27% depending on the concentration. Thus, with the help of the chemical analysis we could determine only the level of soil pollution by HM that does not display their influence on bacterial microflora.

Then we used the MST method for determining the soil state of Almaty city. Firstly we evaluated the quantity of consumed substrates. Basing on the analysis of profiles of organic substrates assimilation by different soil suspension from the samples T.1-3 we determined the quantitative and qualitative differences in their consumptions. Thus the microbial communities of the polluted soils of T.1 out of 47 substrates assimilated only 9 (Table 3).

18 were assimilated by the bacterial community of soil samples T.2: sugars, aminoacids, organic acids; only amylum is referred to the complex polymeric compounds. Microbial community of the soil sample T.3 taken from the park belt, assimilated 30 simple and complex substrates out of 47 (Table 3).

According to the literature data [10, 11] coefficient d, which displays the stability of the community is the most informative one. The results of our investigation showed that the microbial communities of T.1 referred to the critical destabilized system with irreversible damages: parameter d=0.9 i.e. the state of the microbial communities of the soil ecosystems is the worst one near the big traffic artery. These soil samples contained a significant amount of HM (Table 2). The state of bacterial community of the soil samples from the airport (T.2) turned out to be moderately deprimated, the content of HM was close to the maximum permissible concentrations, but it did not exceed these concentrations, index d characterised T.2 as the system which was being influenced by the adverse factor. To the satisfactory systems we can refer the communities of T.3 having d=0.05 and high intensity of organic substrates utilization (Table 4).

The minimum value of coefficient D of the order distribution (stability index) that allows to evaluate the non-stability of a system, was detected in the soil of the sample T.1. The maximum stabilizing activity was observed for the microbial society of the soil sample T.3. The samples of T.2 occupied some borderland position.

Report: Basing on the study of structural characteristics, the acquired results of the soil community state influenced by HM can be characterised as insufficient. The functional (dynamic) characteristics should be determined by means of other methodological approaches, as for example MST method.

During the investigations by MST method we came across two types of systems - normal soil and polluted soil. HM negatively influenced the soil samples of T.1, these systems can be characterised as irreversibly damaged systems which have lost their primary functional unity. Microbial communities of T.2 were averagely influenced by HM, the minimal impact was observed for T.1. Consequently low values of coefficient d are the characteristic of undisturbed communities and the increasing of d is an indicator of the violation of community functioning. The parameters of order distributions coincided with the HM content in the soil samples under analysis and with the biodiversity and microorganisms quantity.

Generally the microbiological methods of microbial communities' state study through their quantity can be used for assessment of soil ecosystem, but these methods take a lot of time and efforts and are less informative. Chemical methods can only detect the level of HM content in the soil. Thus, the defining of the soil state in Almaty city, which was conducted for the biogeneous part of soils with the help of MST method and with more precise indicators of normal soil functioning (SES, d and D) turned out to be much more effective and fast.

The article was prepared within the framework of government grant of Ministry of Education and Science of the Republic of Kazakhstan # 374.

REFERENCES

1. Ladonin, D.V., 2002. Heavy metal compounds in soils - problems and methods of investigations. Soil Sciences, 6: 682-692.
2. Olayinka, A. and G.O. Babalola, 2001. Effects of copper sulphate application on microbial numbers and respiration, nitrifier and urease activities and nitrogen and phosphorus mineralization in an alfisol. Biol. Agr. Hort., 19(1): 1-5.
3. Kelly, J., 1999. Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. Soil Biol. Biochem., 31(10): 1455-1465.
4. Babich, H. and G. Stotzky, 1995. Heavy metal toxicity to microbe-mediated processes: a review and potential application to regulatory policies. Environ. Res., 36(1): 111-137.
5. Gorlenko, M.V. and P.A. Kozhevin, 1994. Differentiation of soil microbial communities with the help of multi-substrate testing. Microbiology, 63(2): 289-293.
6. Mynbaeva, B.N. and M.S. Panin, 2011. Change of metabolic profiles of bacterial communities of soils in Almaty city, polluted by heavy metals. Modern problems of science and education. 3; Date Views 22.08.2011 www.science-education.ru/97-4653.
7. Methodology of measurement of mass content of moving forms of metals, 1990. RD 52.18.269-90. Moscow: publishing of standards, pp: 35.
8. Bloem, J. and A.M. Breure, 2003. Microbial indicators. Bioindicat. Biomonitor., 6: 259-282.
9. Netrusov, A.I., 2005. Practice in microbiology. Moscow: Moscow State University, pp: 608.
10. Garland, J.L. and A.L. Mills, 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol., 57(8): 2351-2359.
11. Insam, H. and A. Ranggerg, 1997. Comparison of Biolog and phospholipid fatty acid patterns to detect changes in microbial community. Abstracts conf. «Microbial communities: Functional versus structural approaches». Berlin, pp: 37-48.